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Priority Toxic Pollutants in Human Urine: Their Occurrence and Analysis

U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards
National Measurement Laboratory
Center for Analytical Chemistry
Washington, DC 20234

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Final Report

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Prepared for
Environmental Monitoring Systems Laboratory
U.S. Environmental Protection Agency
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U.S. DEPARTMENT OF COMMERCE, Malcolm Baldrige, *Secretary*
NATIONAL BUREAU OF STANDARDS, Ernest Ambler, *Director*

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Priority Toxic Pollutants in Human Urine: Their Occurrence and Analysis

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This survey reviews and discusses the occurrence of priority pesticides and industrial chemicals in human urine. An overview of some recent analytical methodology for determination of selected toxic pollutants and their metabolites as they are found in human urine is also presented. The review includes 427 references.

Key words: chemicals; exposure; human; industrial; methodology; pollutants; survey; toxic; urine.

I. INTRODUCTION

The many benefits of our modern developing, industrial society are accompanied by certain existing and new, man-made environmental hazards. These can arise from air pollution, non-ionizing radiation, environmental carcinogenesis, and also from toxic pesticides, the effects of their metabolites, as well as other chemical pollutants. Next to analysis of toxic substances in human blood, plasma, or tissue (1), monitoring of human urine for toxic pollutants is of paramount importance. Human urine is a complex mixture containing hundreds of organic (and many inorganic) compounds (1), and its composition is constantly changing due to changes in diets as well as human environmental exposure. Consequently, addition of one or two new organic compounds (or their metabolites) each year to the array of chemicals known to be present in human urine is not surprising.

The aim of this survey is to assess human exposure to certain pesticides and industrial chemicals, and particularly to discuss the occurrence of such substances in human urine; an overview of some recent methodology for determination of selected toxic pollutants and their metabolites as they are found in human urine is also presented. The priority toxic substances in human urine that are discussed in this survey are: phenol, chlorinated phenols (e.g., mono-, di-, tri-, and penta-chlorophenol), p-nitrophenol, and hydroxybenzene derivatives (e.g., cresol and catechol); also, the herbicides 2,4-D, 2,4,5-T, Silvex, and Dicamba; and certain phthalates, e.g., bis(2-ethylhexyl) phthalate, diethyl phthalate, and others. The literature cited covers, approximately, the period of the past ten years, with emphasis on the most recent references (1975 - mid 1982).

A. Handling of Samples of Human Urine

According to Dr. Robert Purcell, Chief of the Hepatitis Section at the National Institutes of Health (2), urine samples are generally handled in his laboratory with the same precautions as are used for other human samples. He has indicated that transmission of hepatitis A is very unlikely, and is very uncommon in the United States, also that hepatitis B is not transmitted by urine. His studies, involving the inoculation of primates with urine from hepatitis A carriers, did not reveal transmission of the virus. Normal safety precautions are observed in handling these samples, to avoid hand-to-mouth contact in the laboratory. Eating, drinking, or smoking are not allowed. Pipetting by mouth is not allowed; work areas and instruments are cleaned and disinfected after use. Working in a biohazard hood is not required, but such hoods should be used if available.

II. PENTACHLOROPHENOL IN HUMAN URINE (OCCURRENCE AND DETERMINATION)

A. General

Pentachlorophenol (PCP) and its water-soluble sodium salt, sodium pentachlorophenoxide (Na-PCP) are used as biocides. Although they are mainly employed for preservation and treatment of wood, their antimicrobial, antifungal, herbicidal, insecticidal, and molluscicidal properties have led to widespread application of PCP formulations. The literature up to 1967 on the chemistry, toxicology, and environmental residues of PCP has been reviewed by Bevenue and Beckman (3) and by Bevenue et al. (4-6).

A recent discussion on the uses, exposure, toxicology, pharmacology, and environmental impact of PCP can be found in a review by Detrick (7), by Kunde and Bohme (8), by Ahlborg (9), and in a comprehensive book by Rao (10), in a chapter by Dougherty (11), and in a recent Russian book by Fudel'-Osipova et al. (12). The analysis of PCP has been reviewed by Firestone (13), by Dahms and Metzner (14), by Taylor (15), by Thompson (16), by Edgerton et al. (17), by Lakings et al. (18), and in a recent methodology book by Eben (19).

The environmental effect and the human-health risks resulting from the use of PCP as a wood preservative and pesticide have recently been discussed and reviewed (20-23).

The estimated annual production of PCP is 90×10^6 kg world-wide (24); the majority of the material is used as a wood preservative by the lumber industry, and as a fungicide and a bactericide. Commercial preparations of PCP may be contaminated with extremely toxic, chlorinated dibenzo-p-dioxins (7, 25-27).

Potential sources of PCP in human urine can be paper products, beverages and foods; also city water, beef, grain products, and produce (10,28-31) (as a residue from the application of herbicide). PCP in urine can also be a metabolite of such fungicides as hexachlorobenzene (32-34) or hexachlorocyclohexane (35). PCP has been shown (9,36) to be metabolized in mammals to tetrachloro-p-hydroquinone. Evidence has also been presented that both PCP and tetrachloro-p-hydroquinone in urine exist as conjugates with D-glucuronide or as a sulfate adduct (18).

PCP, although moderate in toxicity (8), can be hazardous, as demonstrated by cases in which death resulted from intense exposure (10,11,37); several studies have been conducted relative to dermal and gastric toxicity (38) or toxicity by inhalation (39). PCP is reportedly (40,41) mutagenic. However, in their comprehensive review on the environmental effects of pollutants, Kozak et al. (42) dispute the alleged mutagenic and tumorigenic properties of 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, tetrachlorophenol, and PCP; although some chlorophenols may be embryotoxic. In recent reviews (42,43) it is also pointed out that PCP is the most toxic of the chlorophenol series, but that chronic toxicity of chlorophenols to humans has never been documented. From these phenols, however, 2-chlorophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol have been shown to be carcinogenic in test animals (42-44).

The average, urinary concentration of PCP in non-occupationally exposed individuals is about 20 µg/L (5-7,18,30) or 6-23 µg/L (45); for individuals who live in PCP-treated rooms, the range is 30-150 µg/L (45). The urine of exposed, industrial workers may contain 1.20 - 2.40 mg of PCP/L (14,45); compare, however, additional data (15,18,46), and see also data in Table 1.

It has been reported that PCP (and phenol) in urine can be stabilized and preserved by storage (47) at 4°C or by being frozen (83). It has been reported (48) that urine samples (10-15 mL) can be preserved for up to 15 days when treated with a few drops of saturated, 8-quinolinol sulfate solution. Phenol, perhydrol, or formalin are less effective preservatives. The occurrence of PCP (free or conjugated) (10,18,49,50) in human urine, or of PCP levels found in human urine samples by the EPA Analytical Manual Method (15-17) are well documented. The carbon-13 NMR chemical shifts for pentachlorophenol and pentachloroanisole have been reported (50b).

B. Methods for the Analysis of PCP

Several methods for the determination of PCP in urine have been reported in the literature (13-18). Bevenue et al. (5,6) and Rivers (51) acidified urine, extracted with an organic solvent, and methylated the urine extract with diazomethane prior to electron-capture gas chromatography (EC-GC). Cranmer and Freal (52) partitioned possible interfering compounds into a base prior to acidification, and extracted PCP into an organic solvent. The urine extract was methylated with diazomethane, and analyzed by EC-GC. Shafik et al. (53) determined PCP and other halo- and nitro-phenols in urine by acid reflux, extraction with ethyl ether, derivatization with diazoethane, and separation on a column of silica gel prior to EC-GC analysis.

On the average, 75 percent of the PCP in urine is conjugated; if the conjugate is the D-glucuronide or sulfate adduct (which are the most likely adducts for PCP), hydrolysis to the free compound can be accomplished by enzymatic (aryl sulfatase, β-D-glucuronidase) or acid hydrolysis. The analysis of a hydrolyzed urine provides a measure of the conjugated and unconjugated levels of a compound (18).

Recently, Edgerton and Moseman (54), Edgerton et al. (17,55,56) and Zimmerli et al. (45) found that, prior to derivatization, hydrolysis of a urine sample at 100 °C for 1 hr in a Teflon-lined screw-capped sealed vial, gave a much higher level for biologically incorporated PCP (free and conjugated) than when methods not employing hydrolysis were used. After hydrolysis and extraction, the sample was treated with diazomethane, to produce the methyl ether of PCP, prior to analysis by EC-GC. An acid alumina column as a clean-up system was developed (54,56) to remove interference from the sample extracts, thus allowing detectability of 1 µg/L of PCP (55).

The hydrolysis procedure (45,54) thus permits a highly selective and more quantitative method for the determination of PCP in urine, as may be seen from a comparison of alternative methods, summarized in Tables 1--3.

Table 1: Comparison of PCP Levels Found in Human Urine Samples by Different Methods (54)

Sample	PCP found (ppm)[a] method		
	EPA manual method (17)	Reported methods (51,52)	Hydrolysis method (54)
general population	<0.01	<0.01	0.02
general population	0.02	0.02	0.08
general population	0.01	<0.01	0.04
general population	<0.01	<0.01	0.02
exposed worker	0.54	0.21	3.68[b]
exposed worker	0.41	0.31	1.71

[a]Average of three determinations.

[b]The method presented in this paper yields as much as a 17-fold higher PCP level after hydrolysis than do the other methods used for comparison.

Table 2: PCP Content of Urine of Non-exposed Persons (45)

Urine sample (general population)	PCP Content (µg/L)	
	Hydrolyzed urine	Non-hydrolyzed urine
1	18.8[a]	5.8
2	23.0	6.7
3	11.1	2.3
4	14.3	3.7
5	15.3	5.1
6	19.6	6.1
7	11.4	4.8

[a]PCP level was 3 to 4-fold higher after hydrolysis.

Table 3: Levels of Pentachlorophenol (PCP) (Conjugated and Nonconjugated) in Hydrolyzed, Test and Control Urine Samples (18)

Madison County Health Dept. number	Pentachlorophenol (ppb)[a]		Ratio of Hydrolyzed to Non-hydrolyzed
	Hydrolyzed urine	Non-hydrolyzed urine	
122A - test case	4.5	2.6	1.7
206A - special case	38.8	5.1	7.6
209A - special case	87.6	46.8	2.0
210A - special case	110	50.8	2.2
104B - control	15.5	2.2	7.0
118B - control	6.8	3.5	1.9
204B - control	18.3	4.1	4.5
222B - control	9.4	2.2	4.3
HANES II[b]	<5		

Recovery Values of Pentachlorophenol in Hydrolyzed Urine

Sample	Level spiked (ppb[a])	Level recovery (ppb)	Recovery (%)
Urine blank	--	5.6	--
Urine blank	80	82	93
Urine blank	80	72	83
Urine blank	--	7.3	--
Urine blank	80	83	95
Urine spiked	80	82	94

[a] ppb = parts per billion (ng/mL), sensitivity - PCP, 0.4 ng/mL.

[b] Preliminary data based on the analysis of 4,480 to 4,580 samples collected from the general population via the Health and Nutrition Examination Survey II (HANES II); 81% of the urines showed a positive PCP response, with 11.6% having a trace level; the highest value was 2,670 ppb and the geometric mean was <5.

In another, recent procedure (56), chlorinated phenols in urine are isolated by sorption onto a small column of the macroreticular resin, XAD-2, followed by elution (2-propanol--hexane), concentration, and detection by EC-GC. Results for the human, general-population samples show low-ppb levels of PCP and certain di-, tri-, and tetra-chlorophenols. Gas chromatography (GC) and electron capture (EC) detection (57) have been extensively used for the analysis of chlorophenols in human urine (14,53,58,59).

Recently, a new reference method has been described (59) for confirmation of the presence of chlorophenols in human urine. A hydrolyzed urine sample is analyzed by both gas chromatography (GC) with electron capture (EC) and liquid chromatography (LC) with electrochemical detection (EC); the results compared in Table 4. As shown (Table 4) (59) there is a general agreement between the results obtained by two methods for pentachlorophenol, 2,4,5-trichlorophenol, and 2,4- or 2,5-dichlorophenol. The EC/GC analysis appears to give false positives for 2,4- and 3,5-dichlorophenol while EC/LC gives false positives for 2,3-dichlorophenol and 2,4,6-trichlorophenol. These data support the complementary use of both EC/GC and EC/LC for identification and confirmation of chlorophenols in human urine.

C. Additional Analytical Methods for PCP

Negative chemical-ionization (NCI) (60,61) mass spectrometry (analogous to gas chromatography with an electron-capture detector) (52) is an effective tool for detecting polyhalogenated organics (e.g., chlorophenols) and other toxic compounds in urine (30,31,60-64); it shows high sensitivity (1--10 ng is sufficient to produce a usable spectrum). The procedure generally requires solvent extraction, hydrolysis of urinary conjugates, derivatization, and chromatographic separation prior to NCI analysis. However, in many cases, the NCI method offers the advantages of (a) direct analysis after evaporation, which eliminates the need for derivatization, and (b) mass analysis, which gives information that bears directly on the molecular weight of the residue.

In a recent, analytical procedure (65), PCP is extracted from urine by acidifying the urine sample with citric acid, and then steam-distilling. The distillate is treated with a pH 4.16 buffer, 4-aminoantipyrine, and potassium hexacyanoferrate, and then extracted with xylene, and the xylene extract is measured spectrophotometrically at 570 nm. PCP could be determined around the biological limit of 2 mg/L of urine by using the technique.

Additional analytical methods for PCP in human urine have been reported for occupational (67-70) and household exposure (45).

Table 4: Comparison of Chlorophenol Levels Found in Human Urine by EC/LC and EC/GC (59)

	Chlorophenols (results in ppb)																	
	2,6-Di-		2,5-Di-	2,4-Di-	2,5- or 2,4-Di-	2,3-Di-		2,3,6-Tri-		3,5-Di-		2,4,6-Tri-		2,4,5-Tri-		2,3,4,6-Tetra-		Penta-	
Sample	LC	GC	LC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC	LC	GC
101	<2	81	56	<6	45	51	<1	<10	<1	20	42	<6	2	40	<1	<10	8	10	16
102	3	21	130	<6	117	130	<1	<10	<1	<20	21	<6	4	<10	<1	<10	6	10	11
103	6	31	18	<6	15	<10	<1	<10	<1	<20	9	<6	3	200	<1	<10	6	6	9
104	<2	69	<6	<6	13	<10	<1	<10	<1	<20	6	<6	2	25	<1	<10	4	9	3
105	10	40	160	<6	132	<10	<1	<10	<1	<20	2	<6	5	55	<1	<10	7	7	6
106	<2	36	27	<6	13	<10	<1	33	<1	<20	11	<6	<1	65	<1	<10	3	7	5
108	<2	31	200	<6	156	<10	<1	20	<1	<20	18	30	37	100	<1	<10	9	<5	9
109	<2	50	<6	<6	9	<10	<1	<10	<1	<20	36	<6	4	75	<1	<10	7	<5	14
110	<2	66	250	<6	179	<10	<1	<10	<1	<20	12	<6	<1	100	<1	<10	6	16	13
111	<2	99	20	<6	25	<10	<1	<10	<1	<20	30	<6	<1	100	<1	<10	3	<5	6

III. MONO-, DI-, TRI-, AND TETRA-CHLOROPHENOL IN HUMAN URINE

A. General

Chlorophenols constitute a group of pollutants commonly found in the environment. These compounds arise from degradation or metabolism of pesticides, chlorination of the water supply, and direct introduction from industrial sources (9,10,42,71-75,81). The primary source of human exposure to chlorophenols (except PCP) is likely to be from the degradation of such chemically related phenoxyalkanoic herbicides as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (42). Some of the chlorinated phenols of environmental origin are listed in Table 5 (59), and a comparison of their levels in human urine is given in Tables 6-7.

In addition to PCP (Section II), mono-, di-, tri-, and tetra-chlorophenols may appear in the urine of humans as a result of direct exposure to environmental sources or to pesticides or other compounds that metabolically give rise to chlorinated phenols. Engst et al. (76) identified 2,3,4,6- and 2,3,5,6-tetrachlorophenol, 2,4,6- and 2,3,4-trichlorophenol, and pentachlorobenzene as urinary metabolites from the fungicide hexachlorobenzene (HCB), and Angerer et al. (77) and Koransky et al. (78) found 2,4-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol, and 2,3,4,5-tetrachlorophenol as urinary metabolities from α-hexachlorocyclohexane.

Renner and Schuster (79) reported 2,4,5-trichlorophenol as a new urinary metabolite of HCB. A recent claim (80) that 4-chlorophenol in urine is a metabolite of clofibric acid proved to be incorrect. 4-Chlorophenol is an artifact produced from clofibric acid by proton-catalyzed hydrolysis or thermolysis in the analytical processing of urine (81). The urinary metabolite of chlorobenzene in man has been identified as 4-chlorocatechol (82).

Recently, Edgerton (83) studied the storage stability of chlorinated phenols e.g., 2,5-dichloro-, 2,4,5-trichloro-, 2,4,5,6-tetrachloro-, and pentachlorophenol in urine. A decrease of 40 percent was noted in urine fortified with known amounts of chlorophenols, e.g., 2,4,6-trichlorophenol, and analyzed over a 36-day period with repeated freezing and thawing. A sample-storage technique was presented that allowed for the stability of chlorophenols in urine for over 40 days with little or no decomposition noted. This technique involved subdividing all urine samples into 5-mL aliquots when first received, and keeping them frozen until analysis, to avoid repeated, freezing-and-thawing cycles. The study (83) demonstrated the importance of proper sampling and storage of urine samples when quantitatively analyzing for chlorophenols over an extended period of time. The stability studies of other potentially degradable compounds in urine, e.g., parathion, anilines, and 2,4-D have been described in the literature (84-86). A recent stability study (87) of some environmental compounds such as phthalate esters or polychlorinated biphenyl present in the seawater samples showed that between 2.2 percent and 49.9 percent of organic solutes were lost from the spiked solutions during storage; the loss of the analyte was ascribed to vaporization, degradation or adsorption to the glass container walls.

Table 5: Possible Origins of Various Chlorinated Phenols (59)

Metabolite	Origin	Type of Pesticide
2-Chlorophenol	2,4-D	Herbicide
3-Chlorophenol	PCP	Fungicide
4-Chlorophenol	Chloroxuron	Herbicide
2,6-Dichlorophenol	Lindane	Insecticide
2,4-Dichlorophenol	VC-13	Insecticide
	m-Dichlorobenzene	Fumigant
	2,4-D	Herbicide
2,3-Dichlorophenol	Lindane	Insecticide
	o-Dichlorobenzene	Fumigant
2,5-Dichlorophenol	Lindane	Insecticide
	p-Dichlorobenzene	
	2,4,5-T	Herbicide
3,4-Dichlorophenol	PCP	Fungicide
	o-Dichlorobenzene	Fumigant
	Diuron	Herbicide
3,5-Dichlorophenol	Lindane	Insecticide
	PCP	Fungicide
2,3,4-Trichlorophenol	Lindane	Insecticide
2,3,5-Trichlorophenol	Lindane	Insecticide
	PCP	Fungicide
2,3,6-Trichlorophenol	Lindane	Insecticide
2,4,5-Trichlorophenol	Ronnel	Insecticide
	Tetrachlorvinphos	Insecticide
	Erbon	Herbicide
	2,4,5-T	Herbicide
	HCB	Fungicide
2,4,6-Trichlorophenol	Lindane	Insecticide
3,4,5-Trichlorophenol	Lindane	Insecticide
2,3,5,6-Tetrachlorophenol	HCB	Fungicide
	PCP	
2,3,4,6-Tetrachlorophenol	PCP (impurity)	Fungicide
	Lindane	Insecticide
2,3,4,5-Tetrachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
Pentachlorophenol	PCP	Fungicide
	Lindane	Insecticide
	HCB	Fungicide
	PCNB	Fungicide

Table 6: Accuracy, Precision, and Detection Limits for the Determination of Chlorophenols in Human Urine (77)

Chlorophenol	Nominal value	Actual value		Average recovery	Detection limit
		Mean	Relative standard deviation		
	µg/L	µg/L	%	%	µg/L
2,6-dichlorophenol	174	151	10.1	87	10.0
2,4-dichlorophenol	194	203	8.3	105	8.3
2,3-dichlorophenol	220	231	9.4	105	16.8
3,4-dichlorophenol	151	139	10.1	92	18.6
2,4,6-trichlorophenol	73	70	7.5	96	8.5
2,4,5-trichlorophenol	58	58	5.1	100	14.8
3,4,5-trichlorophenol	66	78	7.7	119	11.0
2,3,4,6-tetrachlorophenol	68	72	4.9	106	4.9
2,3,4,5-tetrachlorophenol	133	156	5.2	117	7.1
pentachlorophenol	127	148	4.4	117	9.0

B. Analytical Methodology

The methodology for the chemical analysis of pesticides and their metabolites, has been reviewed (6,16,88). The most frequently employed analytical procedures for measuring low levels of chlorinated phenols in urine involve solvent extraction and either derivatization (often with toxic, derivatizing reagents like diazomethane) to more chromatographable compounds (35,37,89-93), or determination of the free phenol in various polyester, gas-chromatographic columns (94-97). Recently, reports of analytical methods that use porous, polymer resins as the sorbing agents for the removal of phenols from water (90-100), have been published. The most widely used resin for this purpose is Amberlite XAD-2.

The efficiency of recovery of pesticides (99) and phenols (98,99) from water by using XAD-2 resin or the chemially identical XAD-4 resin has been reported. The free phenols isolated can be separated by using columns of both double support-bonded diethylene glycol succinate (DSB-DEGS) and support-bonded, 1,4-butanediol succinate (SB-BDS) (27-29).

A recent report by Lores, Edgerton, and Mosemann (59) described an efficient method for the confirmation of chlorophenols in human urine by liquid chromatography (LC) with an electrochemical detector. The method was sensitive to chlorophenols at the low-ppb range (as encountered in most general-population samples).

Advantages and disadvantages of liquid chromatography in industrial-medical technology have been discussed (107).

Table 7: Chlorophenols in Human Urine Samples (Results in ppm) (111)

Sample	Detection Method	2[b]	3[b]	4[b]	2.3	2,4[c]	2,5[c]	2,6	3,4	3,5	2,3,4	2,3,5	2,3,6	2,4,5	2,4,6	3,4,5	2,3,4,5	2,3,4,5	2,3,5,6	Penta
	M		10	17					27	14			11	5			8	5		2
1	G						6	31		16				17				5		11
	M	40														6	2			
2	G						45			42				2				8		16
	L				51		56			18					40					10
	M		19	11						11										
3	G						117	21		21				4				6		11
	L				130		130	3												10
	M		24	30					44	13						13		11	3	
4	G						15	31						3				6		9
	L						18	6							200					6
	M								63	60						108				
5	G						13			6				2				4		3
	L														25					9
	M		19						33	18	32	13					21			
6	G						13			11								3		5
	L						27						33		65					7
	M						386		96	45				6						
7	G						156			18				37				9		9
	L						200						20	30	100					
	M																			
8	G						9		36				4					7		14
	L														75					
	M						99									12	4	5		18
9	G						179		12									6		13
	L						250								100					16
	M	25	58						64								2	5		
10	G						25			30								3		6
	L						20								100					

[a]M = liquid chromatography-mass spectrometry; G = electron-capture gas-chromatography; L = electron-capture--liquid chromatography.

[b]Liquid-chromatographic--mass-spectrometric results only.

[c]2,4- and 2,5-Dichlorophenol not resolved by electron-capture gas-chromatography. Electron-capture gas-chromatographic results are reported as 2,5-dichlorophenol -- based on detection by liquid chromatography--mass spectrometry, or liquid chromatography--electrochemical detection, or both.

C. Recent Methodology

An application of pyrolysis gas chromatography-mass spectrometry (PGS-MS) to the study of untreated urine yielded a highly reproducible pattern of peaks that may potentially be used to "fingerprint" metabolic abnormalities. No sample preparation is necessary; analysis can be conducted with 5--15 µL of urine (108).

Relative retention-times of the chlorinated phenols in a 2-pyrrolidinone column, and reversed-phase liquid-chromatography have been reported by Moury and Siggia (109). The study showed that ortho-substituted chlorophenols are not retained as strongly as the corresponding meta and para isomers; also 2,4,6-trichlorophenol is not retained as strongly as the dichlorophenols that are not ortho-substituted (hydrogen-bonding effect).

Recently, Edgerton et al. (56) described the determination of traces of chlorinated phenols in human urine by gas chromatography. The analytical procedure involves extraction of the chlorinated phenols by passing hydrolyzed urine through a column of XAD-4 resin. The chlorinated phenols are then eluted from the column with 2-propanol--hexane. The eluate is concentrated, and analyzed by electron-capture gas chromatography (EC-GC). Recoveries of chlorinated phenols from fortified urine averaged better than 80 percent. Method sensitivity is dependent upon the chlorine substitution and the elution time of the phenol through the gas chromatograph. Sensitivity for di- and tri-chlorophenols is 1 µg/L, and, for tetrachlophenol and pentachlorophenol, 2 µg/L.

In recent work, Angerer et al. (77) described a sensitive, gas-chromatographic method for the simultaneous determination of ten chlorinated phenols that appear in the urine of persons exposed to α-hexachlorocyclohexane (see Table 6). The phenolic compounds in the urinary samples are hydrolyzed in acid, and derivatized with acetic anhydride. This simple treatment permits routine application. The stationary phase (8 percent of DC200 on ChromoSorb GAW-DMCS) possesses a high separation capability for the acetic esters of the chlorophenols. The detection limit lies between 4.9 and 18.6 µg/L and this allows determinations even in the environmentally interesting concentration range. The recoveries determined by using aqueous standards ranged between 87 and 119 percent, and the relative standard deviations were between 4.4 and 10.1 percent. The application of acetic anhydride for derivatization of chlorophenols in urine has been confirmed by another laboratory (18). The simultaneous determination, by gas chromatography, of phenol, 2-chlorophenol, 2,4- and 2,6-dichlorophenol, 2,4,6-trichlorophenol, and 2,3,5,6-tetrachlorophenol in the urine of industrially exposed workers has recently been reported (110); the method does not require derivatization. The phenolic compounds are separated by distillation, and then extraction into isopropyl ether and analyzed by GC. The detection limits in urine are 0.1 mg/L for phenol to 1 mg/L for the di- and tri-chlorophenols. The coefficient of variation for each component was 4 percent. The naturally occurring metabolites cresol and 2-heptanone are also isolated, and can be quantified by this method.

Recently, Wright et al. (111) reported determination of the underivatized chlorophenols in human urine by combined liquid chromatography (LC)-mass spectrometry, and selected-ion monitoring. The urine samples were hydrolyzed by the method of Edgerton et al. (56) and LC was performed in a LiChrosorbdiol column (UV detector at 210 nm); the separation of chlorinated phenols is illustrated in Fig. 1 (111).

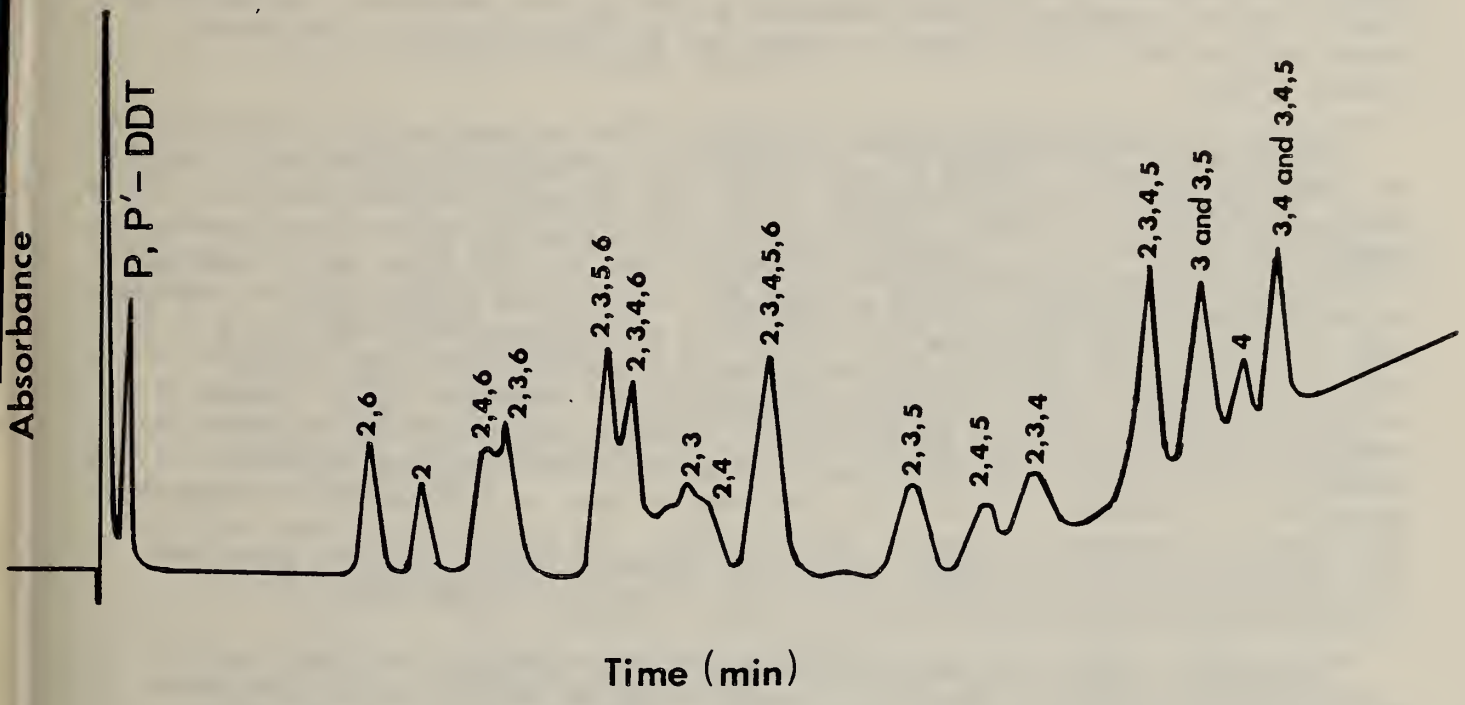

Figure 1. Chromatography of chlorinated phenol standards (250 ng each) on LiChrosorbdiol column. UV detector at 210 nm.

The study of chlorophenols in human urine was approached by selected-ion monitoring because of the large number of interfering compounds. The results of a selected-ion monitoring--LC--mass spectrometric study of ten individual, human-urine samples are presented in Table 7. The results obtained from the same extracts by electron-capture GC and electrochemical detector LC are presented in the same Table for comparison. The specific details of the last two methods are reported elsewhere (54-56). In many cases, the results are in reasonable agreement; in others the disagreement is distinct. The EC/GC method, by the nature of the detector, has a low sensitivity for monochlorophenols; the EC/LC method requires a buffered mobile phase and does not permit solvent gradient, and the LC-MS method requires two 23 min data collection runs per sample. Each of the three methods was capable of low-ppb detection when tested against mixtures of standards. When urine extracts were examined, severe background-interference generally limited mass-spectral detection to 5--25 ppm. Each urine sample run was either preceded, or followed, by analysis of a standard mixture containing 0.2 ng of each of the chlorophenols per mL. The method (111) could thus detect 19 chlorinated phenols in human urine in 2--23-min runs, with a lower detection limit of 2--10 ng. In absolute terms, however, the mass spectrometer is less sensitive than either the electron capture detector, or the electrochemical detector, but it is the most selective of the three.

IV. PHENOL, p-CRESOL, CATECHOL, AND RELATED HYDROXYAROMATICS IN HUMAN URINE

A. Phenol (General)

The principal, simple phenols reported to be present in human urine are phenol, p-cresol, catechol, resorcinol, and hydroquinone (quinol) (112-114). However, as recently found by Spiteller and Spiteller (115) and others (116), human urine is a complex mixture containing ∿500 acidic compounds (including phenol and p-cresol), and 2/5ths of these can be characterized by their mass spectra (115).

Phenol is a normal constituent of urine. For the most part, it is found in the conjugated form -- either as the sulfate or as the glucuronide (117); very little free phenol is found in human urine. Normal total-phenol level in human urine is ∿50 mg/L (112,118); however, this value may vary with exposure, or with the methods of measurement (119-124). Phenols in urine may be derived not only from the dietary intake of proteins, fats, such smoked foods as smoked meat, and water, but also from a wide variety of exogenous sources (e.g., tobacco smoke, mouth washes, ointments, and analgesics) and drugs [phenyl salicylate (salol)(125), zinc phenylsulfonate, and phenobarbital]. Exposure to benzene (126,127), which is of particular interest because of its known hemopoietic toxicity (128), may give rise to the formation, and increased excretion, of phenols (112,113) and this may be used as a measure for assessing the degree of exposure of humans 118,126-129). The mechanism of this relationship is that the liver oxidizes benzene to phenol, and subsequently detoxifies the phenol by conjugation with either sulfate or D-glucuronic acid.

Industrial organic solvents are commonly used nowadays, and they are potentially injurious to health. It is specific for this group of compounds that, according to the latest findings, it is often not the toxicological compounds themselves but their metabolic products that are dangerous to health (130).

In a cross-sectional investigation (131), the results of 6126 toxicological urine tests of persons exposed to such industrial solvents as benzene or toluene have been statistically evaluated. The solvent metabolites from benzene → phenol, and from toluene → hippuric acid and p-cresol, have been defined as hazards in occupational medicine.

Williams (112,113) reviewed the metabolism of phenols. The relative amounts of these vary with the individual, and abnormal patterns of phenol excretion by the kidney may be due to antibiotic intake, gastrointestinal disorders, and liver and kidney diseases (132).

a. Preservation of urine samples containing phenol

The urine samples to be analyzed for both free phenol and total phenol were stored at -20 °C in sterilized, 20-mL bottles, or polyethylene, screw-capped jars, within 30 min after collection, and were thawed immediately before analysis. Control samples showed no change after several weeks (132). In a more-recent report (133), the urine specimens containing phenol were collected in polyethylene bottles. Whereas no specific treatment appeared necessary to preserve the integrity of the sample, 1 mL of 10 percent copper sulfate solution

was added to inhibit bacterial activity, and samples were stored in a refrigerator at 4 °C until analyzed. Storage (47) at 4 °C, or freezing (83), is recommended for preservation of urine samples, as reported earlier in this text. Urine samples selected for total-phenol analysis were preserved by collecting them in 1 M hydrochloric acid and storing them at -20 °C until analysis (134). Urine samples were also preserved by adding a drop of chloroform to prevent microbial growth (135).

B. Analysis of Phenol in Human Urine

The quantitation of total urinary phenol has only been practical from the clinical standpoint for ∿50 years. Some early, and some recent, methods of urinary phenol analysis had been limited to colorimetric methods using either coupling of a diazonium chloride with phenol (136,137,185), or the Gibbs reagent (indophenol test) (138,160), or color-producing reagents, e.g., 4-amino-antipyrene produces a stable color with phenol (520 nm) if the pH is kept (139,140) at 10.0 ±0.2. These methods, however, lack specificity, as the cresols present in urine interfere with development of the color. The colorimetric methods of urine analysis were reviewed, and criticized (132,141). Also, analyses for urine phenol sulfates, either by paper chromatography (142) or paper electrophoresis (142-144), are inadequate, because urine contains large proportions of salts and polyols (145).

Recently, however, Ragan and MacKinnon (146) used paired-ion, reversed-phase, liquid chromatography (LC) for both qualitative and quantitative analysis of phenol sulfates in these complex, biological mixtures; the urine mono- and di-sulfate of resorcinol have also been determined by this method. The range of detection (254 nm absorption) was from 30--125 ng to 5--10 µg, depending on the compound (e.g., phenol sulfates). The paired-ion, reversed-phase, method (146) thus constitutes a considerable improvement over paper-chromatographic and paper-electrophoretic analysis of phenol sulfates, particularly those occurring in crude extracts of urine. Normal (polar), phase-adsorption LC (147-149) usually leads to poor retention characteristics and poor resolution of such strongly ionized compounds.

The conditions used for hydrolysis of conjugated phenols vary widely (150), and range from (132,135,151) refluxing urine for 30 min after adjustment of the pH to 1 to refluxing for 60 min with an equal volume of concentrated hydrochloric (135,150) or sulfuric (152) acid. Sulfate-conjugated phenols are hydrolyzed by acid at 93--95 °C at least 50 times as quickly as glucuronide-conjugated phenols (150,153). The hydrolysis of the conjugated phenols can also be performed enzymically by using a mixture of arylsulfatase or β-D-glucuronidase (154).

a. The Van Haaften--Sie gas chromatography method for the measurement of phenol in urine

In 1965, Van Haaften and Sie (155) developed a gas-chromatographic (GC) technique that enabled determination of the phenol concentration in urine without interference by cresol. Their technique (155) requires special injection-equipment and, furthermore, the volume of the sample injected must be measured with great accuracy. The urine sample was mixed with an equal volume of phosphoric acid, and injected into the gas chromatograph. Conjugates were

hydrolyzed in a precolumn containing coarse-glass powder, and the phenol was separated from other urinary components in an analytical column, and detected by flame-ionization. A few parts per million of phenol in urine could be detected, and this was a significant break-through in the analysis of urinary phenol. A recent, critical appraisal of this method was presented in a paper by Baldwin et al. (156). The authors (156) found that the method gives over 80 percent (actually 89 percent) of the response expected for conjugates of phenol. However, they found that the response was strongly dependent on instrumental conditions, and that safeguards must be built into the method, in order to ensure that hydrolysis of conjugates is satisfactory (or complete). Despite some drawbacks, the Van Haaften-Sie method has found wide application as a measure of benzene exposure in humans; the method has been modified by several research groups (125,128,132,134,135,154,157-164).

The use of an appropriate, internal standard in GC analysis is essential if reliable results are to be achieved with the Van Haaften--Sie method (158). However, an internal standard was not used originally and chromatograms frequently showed a relatively high background, making quick chromatographic analysis difficult. This method has been modified by introducing an internal standard such as valeric acid (161), benzyl alcohol (154), o-cresol (135), 2-ethylphenol (133), 2,5-xylenol (162), or 2,4-dichlorobenzene (119). A standardized procedure for a GC analysis of phenol in urine has been suggested (135), and is detailed next; the steps involve hydrolysis of conjugates, addition of o-cresol as the internal standard, extraction with ether, and GC analysis.

Suggested procedure for a GC analysis of phenol in urine (135)

1. To the sample of urine (containing 1--200 μg of phenol) in an ampoule, add sufficient hydrochloric acid to give a final strength of 5 mol/L, flush the ampoule with nitrogen, and seal.*

2. Heat the ampoule at 100 °C for 30 min; then cool.

3. To the opened ampoule, add a known amount of o-cresol, estimated as far as possible to be similar in amount to the phenol in the sample, and mix.

4. Transfer to a small separatory funnel, rinsing with water as necessary, and extract the mixture with about twice its volume of ether.

5. Centrifuge, and transfer the ether layer to a tapered, evaporating tube by means of a Pasteur pipette.

6. Evaporate the ethereal solution to ∿50 μL on a water bath at 30 °C.

7. Inject 1--5 μL of the concentrate on to the GC column (4 ft x 1/2 in) containing 3 percent of OV 25 on Chromosorb W-HP (80--100 mesh) at 100 °C.

*Screw-cap vials are more convenient, see Ref. (56), Part II.

However, gas-chromatographic methods are not without their problems, mainly at the sample-preparation stage. Firstly, the samples must be hydrolyzed to decompose the glucuronide and sulfuric ester of phenol. This step is usually conducted by heating with acid, and it carries the twin dangers of incomplete hydrolysis and loss of the very volatile phenol from heated solutions. The hydrolysis may also be performed enzymically. Secondly, the phenol must be extracted from the hydrolyzed sample: simple extraction usually recovers only 50-60 percent of the available phenol, and even salt saturation of the aqueous layer increases extraction efficiency (52,129) to only 80 percent. Unfortunately, much extraneous material is simultaneously extracted, giving chromatograms showing interference and rising baselines. Finally, the phenols are often derivatized [e.g., trimethylsilyl (TMS) or acetate derivatives] before analysis, requiring further, labor-intensive manipulations (132,133,164). According to Fell and Lee (164), acetates are conveniently prepared following extraction by treating dilute solutions of monohydric and dihydric phenols at room temperature with acetic anhydride in the presence of 4-(dimethylamino)pyridine (which is a far more powerful catalyst than pyridine). The phenol acetates are then analyzed by GC using wall-coated, glass-capillary columns.

A rapid GC microdetermination of urinary, volatile phenols (phenol, p-cresol, and 4-ethylphenol), following hydrolysis with hydrochloric acid and steam-distillation, has recently been described (134). A determination of phenol and catechol with immobilized enzymes has recently been reported (164a).

b. Phenol in urine: additional sources; determination, and methods of analysis in brief

Phenol in urine as a result of industrial exposure to benzene has been reported from many laboratories throughout the world. One study (165) showed that, within 4 h, an average of 40 percent of the benzene absorbed was excreted as phenol. Other studies included detailed urinary monitoring (166), benzene metabolites (167), and benzene exposure versus urinary-phenol level (129,168). Exposure to benzene and the presence of its metabolite, phenol, in urine has also been reported in workers in the shoe industry (170), the oil industry (171,172); in workers in close contact with oil-shale tars (173) or shale chemical-products (174), or in workers in contact with phenolic resins (175), caprolactam (176), or plastics (176) production, and in filling-station personnel (177).

Additional methods of analysis of phenol in human urine include GC determination of phenol and p-cresol; the values found in urine were 2.9 mg/L for phenol and 3.3 mg/L for p-cresol (178); the values for phenol and p-cresol, however, were considerably lower than reported (112,118,120,121,182). Also, phenol in urine was determined by thin-layer chromatography (t.l.c.) (179,180), or t.l.c.--color reaction (using 2,6-dichloroquinone-4-chloroimide as the color reagent) (179); also, by derivatization (dansyl derivatives) followed by high-speed GC (181-183), or as direct separation as phenyl glucuronide and phenyl sulfuric esters (via multiple, liquid--liquid partition) (184). Additional procedures involved the diazotization of p-nitroaniline, and subsequent coupling of the diazonium chloride with urine phenolic compounds (185); application of mass spectroscopy (186,187); liquid, thermal diffusion (139); multicomponent GC (188-191) or LC (182) analyses; and use of a special color reagent (involving sodium tungstate) (192,193).

C. Cresol, Catechol, Hydroquinone, and Other Benzenediols (Polyhydric Phenols)

a. Cresols

The presence of cresols in human urine after exposure to toluene was first suggested by Laham (194). Further insight into the metabolism of toluene in man was gained by detailed investigations using elaborate, analytical methods, e.g., liquid chromatography (LC) (195) and GC (182,183). o-Cresol was established as a metabolite of toluene, and a detailed comparison between normal persons and an exposed group were presented by Angerer (183), who stated that ∿0.05 percent of the toluene retained was metabolized to o-cresol.

Experimental evidence on cresol in human urine following exposure to toluene has recently been reported by Woiwode and Drysch (196). Ten healthy men were exposed, at rest, to ∿200 ppm of toluene in the air and concentrations of toluene in blood and urinary metabolites were measured with time following exposure. The results of this study are summarized in Table 8. Hippuric acid, o-, m-, and p-cresol, and phenol were detected by capillary GC, thus confirming formation of these metabolites of toluene. Also, as shown in Table 8, the mean value for all metabolites in the urine, except phenol, increased after 4-h exposure; notable also was a dramatic increase in the toluene concentration in blood for the same period of time, with practically no change in density of urine samples.

Using modern, analytical methods, e.g., capillary column, flame-ionization GC, or GC-MS, o-, m-, and p-cresol in human urine were determined in industrial (printing) (182,183,196-199) or refinery (200) workers, and in occupational (197) or experimental (196) exposure to toluene. In one practical procedure (201), a sample of urine was acidified with 0.5 mol/L HCl to pH 5.5--6, followed by extraction of phenol and cresol into ether, clean-up in an activated-alumina column, and flame-ionization GC using Carbowax--cyclohexanedimethanol succinate on Chromosorb W, and nitrogen as the carrier gas. The column temperature was 170 °C. At 0.2--1.4 mg/L of phenol and 1.4--3 mg/L of cresols, the recovery was 90--96 percent.

Colorimetric determination of hydroquinone (202) or catechol (203) in urine was achieved by using specialized reagents, e.g., 3-methyl-1-phenyl-5-pyrazolone (antipyrine reagent, red color of the complex measured at 520 nm) (202) or the Gibbs reagent (2,6-dichloroquinone chloroimide, red color of the complex measured at 510 nm) (203).

GC (204) or GC-MS (205) analysis of alkylphenols, alkylcatechols, and polyphenols in the urine of exposed workers (204), or in uremic serum (205), has recently been reported.

Table 8: Common, Mean Values for Toluene in Blood and Urinary Metabolites from Exposure Experiments (196)

	Before exposure	After 4 hours' exposure	4 hours after exposure	20 hours after exposure	44 hours after exposure
Toluene in blood (g/L)	10	755 3	58 4	21 9	
Hippuric acid in urine (g/L)	0 823	4 208	1 663	1 074	0 680
o-Cresol in urine (mg/L)	0 159	1 603	1 400	0 495	0 130
m-Cresol in urine (mg/L)	0 2*	0 570	0 599	0 527	0 2*
p-Cresol in urine (mg/L)	31 206	39 590	40 968	39 965	40 275
Phenol in urine (mg/L)	4 869	3 483	4 017	6 900	5 033
Density of	1010 8	1007 7	1011 4	1011 8	1014 8

*Below detection limit

b. Benzenediols (polyhydric phenols)

There are many methods for determining monohydric phenols in urine, but at present only a few quantitative procedures exist for the dihydric phenols. For semi-quantitative measurements by paper chromatography, the phenols are usually extracted from neutral urine with organic solvents. Using more sensitive and specific GC methods for quantitation, Bakke and Scheline (158) found this method of extraction to be inefficient and irreproducible; they extracted acidified urine with ether, and then back-extracted the ether layer with aqueous sodium hydrogencarbonate; this procedure gives poor recoveries of the dihydric phenols, but is, nevertheless, the most satisfactory method available. Fell and Lee (164) re-investigated, and improved, the extraction of phenols from human urine; they also developed a new method for derivatization (by preparing the acetates). The urinary monohydric and dihydric phenols as acetates (or trimethylsilyl derivatives) are then analyzed by capillary GC. GC of underivatized dihydric phenols (158) is somewhat difficult.

c. Analysis of biological mixtures by negative chemical ionization mass spectrometry

A recently developed alternative to chromatography--mass spectrometry for the analysis of mixtures is direct analysis in a two-stage, mass spectrometer (206). The first stage functions as an ion separator, and the second, as an ion identifier (207). Recently, McClusky, Kondrat, and Cooks (61) and Levsen and Schulten (208) developed a direct, mixture analysis by mass-analyzed, ion kinetic energy spectrometry (MIKES), using negative chemical ionization (NCI). The NCI/MIKES method (61) is particularly useful for direct analysis of such complex biological mixtures as are found in human urine, e.g., mono- and dihydric phenols, hydroxybenzoic acids, and D-glucose. Because mass analysis does not separate isomers (e.g., o- and p-hydroxybenzoic acid), the MIKE spectrum of $(M + H)^+$ and $(M - H)^-$ fragment-ions offers important structural information. The detection limit is lower in the MIKES method (sensitivity is better than 10^{-10}g) than in conventional, mass spectrometry, and this is accounted for by the decreased chemical noise in MIKES.

V. p-NITROPHENOL IN HUMAN URINE

A. p-Nitrophenol, a Metabolite of the Pesticide Parathion

The methyl- and ethyl-parathions, i.e., O,O-dimethyl- and O,O-diethyl-O-p-nitrophenyl phosphorothionates, respectively, have been among the most widely used insecticides in U. S. agriculture during the past quarter century. The ethyl form has accounted for a large proportion of poisoning among workers exposed to insecticide-treated citrus and cotton (209,210). These incidents demonstrated a need for improved protection of agricultural workers exposed to organophosphate pesticides (209).

It has been reported (211) that, in California in 1977, methylparathion, followed by ethylparathion, were the two most used organophosphorus pesticides. Exposure to methyl- or ethyl-parathion (or similar organophosphorus pesticides) leads to excretion of p-nitrophenol (PNP) in human urine; other metabolites of

parathion, such as alkyl phosphates, have also been found in human urine, and their analysis provides a measure of the extent of exposure. A recent survey by Kutz et al. (72) showed that residues of pesticides and their metabolites can be found in various tissues and body fluids of the general population of the United States, and this is indicative of the environmental distribution of these chemical compounds. For example, Table 9 summarizes the result of the analysis of specimens from a pilot human urine study for the years 1970 -- 1974; alkyl phosphate and phenolic derivative were both detected in urine samples (see also Tables 11-12). Interestingly, from this survey, it may be seen that the pesticides 2,4,-D or Silvex, or the metabolites 3,5,6-trichloro-2-pyridinol, or 2,4,5-trichlorophenol, were not detected during the 1970 --1974 pilot-study.

Table 9: Summary of Pesticides and Their Metabolites Detected in 267 Human Urine Samples (72)

Pesticide or its metabolite	Percent positive	Percent of samples $\geq$ 0.1 ppm
Dimethyl phosphate (DMP)	76.7	2.2
Diethyl phosphate (DEP)	94.0	3.4
Dimethyl phosphothionate (DETP)	70.8	7.9
Dimethyl phosphodithionate	38.2	4.5
Diethyl phosphodithionate (DEDTP)	0.4	0.4
Malathion	0.4	0
Pentachlorophenol	96.3	1.5
Alpha-naphthol	10.9	2.2
Paranitrophenol	6.7	1.9
2,4,5-T	1.5	1.5

The polarographic determination of p-nitrophenol, as a urinary metabolite of parathion and similar pesticides, has recently been reported (239). The measurement was conducted by reduction of the polarographically active, nitro group at a dropping-mercury electrode; the recovery was 98--100 percent. The method permits determination of p-nitrophenol without extraction from urine. The application of pulse polarography to the determination of p-substituted nitrobenzenes (e.g., PNP) and other nitrogen-containing organic compounds, and their potential metabolites, has recently been described (240). The sensitivity of pulse polarography was 0.05 mol/L for nitro compounds, and was decreased ~10-fold in the presence of urine. PNP was determined directly while spiked in urine.

An enzymic procedure for the determination of pesticides in urine has also been described. This new, competitive-assay procedure, namely, enzyme-linked, immunosorbent assay (ELISA), has been developed for the analysis of pesticides, using parathion as the model compound (241). Parathion was reduced to its amino derivative, and this was diazotized, and coupled to bovine serum albumin

(BSA). Approximately 21--23 aminoparathion residues were conjugated to each molecule of BSA. The ELISA procedure was performed by utilzing the antiserum produced, which exhibited high specificity for parathion. Various pesticides (methylparathion, diazinon, dimethoate, and carbaryl) and a metabolite of parathion (p-nitrophenol) showed inhibition of the antiserum. The lower limit of detection of parathion by the ELISA procedure was found to be 5.0--10 ng/mL (0.005--0.010 ppm) in human serum, human urine, and bovine serum.

Correct diagnosis in parathion poisoning depends on the history of exposure to parathion, and on association with the characteristic signs and symptoms. Urinary p-nitrophenol, a metabolite of parathion, can be detected as early as one hour, and as long as 259 hours, after parathion ingestion. The urinary p-nitrophenol of 23 patients with acute, oral parathion poisoning was determined (212). The concentrations of p-nitrophenol in urine in mild parathion poisoning ranged from 1.4 ppm to 10.5 ppm with an average value of 3.9 ppm; in severe parathion-poisoning, the value was >26.7 ppm (212). The toxicity of the pesticide has been inferred from the analysis of a parathion-suicide victims; their urine contained 6.3 ppm of PNP by liquid chromatography, and 6.2 ppm by a confirmatory, spectrophotometric method (213). p-Nitrophenol and p-aminophenol are excreted in the urine of workers involved in the production of nitrobenzene, particularly in cases of acute poisoning (214).

B. Analysis of p-Nitrophenol in Human Urine

The urinary level of phenolic compounds may be the key for establishing an index of exposure to pesticides containing this moeity as a readily hydrolyzed or metabolized portion of the molecule. p-Nitrophenol is excreted entirely as glucuronide and sulfate conjugates in mammals, including humans (215-217). PNP can be readily detected and quantified in biological samples by virtue of its strong, visible absorption at 390 nm. This property makes PNP an excellent substrate for the study of phenolic-conjugation enzymes. Thin-layer chromatography (217,218) and anion-exchange, liquid chromatography (219) have been reported for separation of PNP conjugates; however, quantitation of the separated compounds requires either time-consuming procedures (217,218) or use of a solution of extremely high ionic-strength (mol/L KCl) (219).

The behavior of p-nitrophenol and p-nitrophenyl D-glucuronide with mineral acid has been investigated (220). With 33 percent sulfuric acid, ~93 percent of the glucuronide was hydrolyzed when the solution was heated in an open vessel for 15 s. With 6 percent hydrochloric acid, only ~65 percent of the conjugated p-nitrophenol was converted into the free form. No loss of PNP occurred when free PNP was treated under the same conditions. Studies of the hydrolysis of PNP conjugates, and determination of free PNP in urine, have been reported (221-228).

p-Nitrophenol as a human-urinary metabolite is generally detected and measured by the multiphenol method of Shafik et al. (53) down to levels of 10 ng/mL. The method measures PNP as p-ethoxynitrobenzene. Analyses by this method have been conducted for a number of years, in order to determine the extent of invasion by PNP residues in the general population. The method (53) is based on electron-capture GC (EC/GC) of ethyl ether derivatives of phenols.

The procedure involves acid hydrolysis, extraction, derivatization (diazoethane), silica gel chromatography, and EC/GC. A mixture of ten phenols and three phenoxy acids, namely 2,4-D, 2,4,5-T and Silvex can be determined in one sample. The sensitivity of this method is comparable to that of recent findings (111); for example, a limit of detection is o.01 ppm for 2,4,5-trichlorophenol, 0.01 ppm for pentachlorophenol and 0.02 ppm for p-nitrophenol (56).

The analysis of other metabolic products of parathion, e.g., alkyl phosphates, has also been explored. Alkyl phosphates are metabolic end-products of parathion and all other organophosphate pesticides, and may therefore be useful in evaluating exposure to many more organophosphates than those yielding p-nitrophenol (229-231). Urinary O,O-diethylphosphorothionate (DETP), a primary alkylated metabolite of ethylparathion (229), has been shown (85) to be unstable in urine, even when the sample is kept at -18 °C. Acid hydrolysis of DETP, to give ethyl phosphate, is used in a procedure for the determination of the pesticide by Shafik et al. (232). In this procedure, acidified (hydrochloric acid) urine is autoclaved, extracted with 1:1 acetonitrile--ether, and the material in the extract is derivatized with diazoethane. Chromatography on a column of silica gel is used for clean-up, and fractionation, of the alkyl phosphates. A gas chromatograph equipped with a flame-photometric detector in the phosphorus mode is used to quantitate the alkyl phosphates. Improved methodology based on resin absorption (ion-exchange chromatography) of urinary alkyl phosphonates has been developed by Lores and Shafik et al. (229-233). Recently, Bradway et al. (230) effectively extracted the alkyl phosphates in urine, as an ion-pair with a lipophilic, quaternary ammonium cation, into a nonpolar solvent such as dichloromethane containing an alkylating agent [e.g., (pentafluorophenyl)methyl bromide].

C. Recent Methodology

A high percentage of the chemical compounds encountered in the environment are eventually excreted in the urine as various polar metabolites. Therefore, indices of exposure to these compounds may be established by monitoring the levels of urinary metabolites. Because many of the metabolites may be classified into one of several broad categories (e.g., phenols, anilines, and alkyl phosphates), a few fundamental methods permit the monitoring of a large number of metabolites (230). Detection of p-nitrophenol as a urinary metabolite may be related to pesticide exposure; for example, exposure to parathion or its homologs (16,211,234).

Recently, several methods have been developed for confirmation of the presence of p-nitrophenol as a human urinary metabolite. According to Kirby et al. (235), p-ethoxynitrobenzene (as reported by Shafik et al. (53)) in a benzene or hexane extract is reduced with aqueous chromous chloride to p-phenetidine, which is then converted into the amide by reaction with heptafluorobutanoic anhydride. Heptafluorobutano-p-phenetide is then determined by GC in an OV-1 column, using a ^{63}Ni detector. The method was applied to urine samples containing 10 ppb of PNP.

Liquid chromatography (LC) with UV detection (254 nm) has been reported for the determination of PNP in urine (236). Recently, Ott (213) improved this procedure; acid hydrolysis, steam distillation, and LC separation (UV detector) of urinary pesticide-metabolites were performed automatically. At the highest rate, samples were analyzed every 24 min with a limit of detectability of 1--2 ppb of PNP in urine.

Recently, a rapid, sensitive method has been developed for the simultaneous determination of PNP and its glucuronide and sulfate conjugates in biological samples (e.g., urine) by using reversed-phase LC (237). The mobile phase, consisting of 10 mmol/L K_2HPO_4, pH 2.75, containing 20 percent of acetonitrile, provided excellent retention, and separation, of PNP and its D-glucuronide and sulfate conjugates.

A simple, rapid spectrophotometric method for the determination of urinary p-nitrophenol has recently been described (238). The urine sample (10 mL) was mixed with HCl (1 mL), and 25 mL of an extractant (consisting of 4:1 petroleum ether--ethyl ether and 1 percent of pentyl alcohol) was thoroughly shaken for 3 min. The ether phase was washed with water (∿20 mL) and mixed with 14 mL of 3 mol/L NH_4OH, 0.7 mL of 1 percent o-cresol, and 1.4 mL of 1 percent $TiCl_3$, and the reaction complex was measured spectrophotometrically at 620 nm. The reproducibility was good, and recovery of added PNP was 92--100 percent. A sample of PNP (>30 μg) can be detected.

VI. HERBICIDES (2,4-D*, 2,4,5-T**, SILVEX***, DICAMBA****, AND OTHERS) IN HUMAN URINE

A. Introduction

The chlorophenoxy acid herbicides, e.g., 2,4-D, 2,4,5-T, Silvex, and Dicamba, are widely used in agriculture, commerce, and homes in order to control terrestrial and aquatic, broadleaf weeds (242,243). The herbicides 2,4,-D (244) and 2,4,5-T (245,246) have played an important role in improving the forest and food-producing capability of the United States during the past twenty years. Their effectiveness in controlling a wide spectrum of broadleaf, woody plants, and their high rate of degradation when applied to soil, have allowed these herbicides to be widely used (247). The world-wide consumption of these herbicides is now enormous (12). In Sweden, $\sim 2 \times 10^5$ kg of 2,4-D and 2,45-T are used annually in forestry (248). On the Canadian prairies, $\sim 5 \times 10^6$ kg of 2,4-D formulations are applied annually for weed control (249). Agent Orange (a herbicide containing equal parts of 2,4-D and 2,4,5-T) was used extensively in Vietnam as a defoliant (250). Due to widespread application of the herbicides, the environment contains a significant amount of 2,4-D (12,16,23,42,43,251-254); small amounts of 2,4-D have been found in drinking

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*(2,4-Dichlorophenoxy)acetic acid (2,4-D)

**(2,4,5-Trichlorophenoxy)acetic acid (2,4,5-T)

***2-(2,4,5-Trichlorophenoxy)propanoic acid (Silvex)

****3,6-Dichloro-2-methoxybenzoic acid (Dicamba)

water (≤0.04 µg/L) and marine environments (≤1.0 µg/L) (244,255). The solubilities of 2,4,-D and 2,4,5-T in water at 25 °C are 890 and 280 ppm, respectively (256); the appreciable water-solubility of 2,4-D makes derivatization necessary prior to analysis.

Studies with humans who have ingested 2,4-D indicated that the herbicide is first found in the blood plasma, whereupon it is excreted into the urine at a rate dependent on the concentration in the blood (257-259). The intake of 2,4-D, 2,4,5-T, Silvex, or Dicamba can be through inhalation, absorption through the skin (260-262), or other exposure (263); the herbicides are known to have relatively short half-lives of retention (244,264,265) and are excreted largely unmetabolized in urine within 24--48 h (53,266-268); the pesticide chloropyrifos, however, metabolizes in human urine. It is known that 97 percent of any absorbed 2,4,5-T is excreted within seven days (269). Trichlorophenol, a known metabolite of pentachlorophenol (PCP), is also a probable metabolite of 2,4,5-T and other trichlorophenoxy acid herbicides (269,270); however, this assumption needs experimental verification.

The rapid elimination of 2,4-D has been attributed to renal, proximal-tubular secretion (244,271). For this reason, urinalysis is useful qualitatively, and quantitatively, for determining occupational and extraneous exposure to this herbicide.

B. Toxicity of Pesticides

The "Atlas of Cancer Mortality" (272) provides the strongest evidence yet available for the association of human cancer with environmental factors; human mutagenicity may be associated with exposure to 1,2-dichloroethane (273) and human fertility has recently been shown (274) to be adversely affected by exposure to PCP, trichlorophenol, hexachlorobenzene, and many other toxic substances.

Various toxicological studies have been conducted with 2,4-D, 2,4,5-T, and other herbicides and pesticides during the past twenty-five years (72,248,262, 275,276-279). A concerted effort has been made to minimize the level of an extremely toxic impurity, namely, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), in commercial 2,4,5-T (245). The herbicide 2,4,5-T currently contains less than 0.01 ppm of TCDD (280). The extreme toxicity of 2,3,7,8-TCDD necessitates monitoring for it at parts per trillion (10^{-12} g/g) levels in food (281). The occupational exposure to polychlorinated dioxins and dibenzofurans has recently been reviewed (282). The toxicity and metabolism of pesticides in animals and humans is the subject of two recent reviews (72,283) and a recent book (284). In his assessment of the toxicity of 2,4,5-T and related agricultural chemicals, the author of the review (283) suggested that the most useful approach to re-evaluating the presumed, potential risks of these chemicals would be a combined experimental and clinical study. Analysis for p-nitrophenol in human urine is recommended for parathion; the tentative, toxic level for p-nitrophenol is 100 µg/L; for organochlorine pesticides, the proposed toxic level ranges (283) from 20 to 500 µg/100 mL. No profound effects on animals (261,262) or humans (278) have been observed on oral administration of small quantities (3 mg/kg/day) of 2,4,5-T.

C. Toxic Substances in Human Urine

A three-year study to assess the exposure of the general population of the United States to residues of selected pesticides and their specific metabolites through analysis of human urine has been presented by Kutz et al. (71,72), and is briefly summarized (72) in Tables 10--12. The multi-residue approach used for analyzing the samples of human urine permitted the detection of the many chemicals shown in Table 10; the pesticidal origins of these chemicals are also presented. The results of the urine analyses for pesticide residues and metabolites are shown in Tables 11 and 12. Among the pesticides detected, pentachlorophenol (PCP) is ubiquitous, as it has been found in 85 percent of the urine samples analyzed (maximum concentration, 193.0 ppb) (see Table 11). The next most-abundant phenolic residue in human urine is p-nitrophenol (113.0 ppb), followed by the metabolite 3,5,6-trichloro-2-pyridinol (31.7 ppb) and 2,4,5-trichlorophenol (32.4 ppb); very little Silvex (3.2 ppb) and only traces of 2,4-D and 2,4,5-T were found (Table 11). The most abundant dialkyl phosphate residues in human urine were such metabolites of parathion as diethyl phosphothionate (DETP) (900 ppb) and diethyl phosphate (DEP) (420 ppb), followed by dimethyl phosphothionate (DMTP) (360 ppb) (see Table 12). The herbicides 2,4-D, 2,4,5-T, Silvex, and Dicamba are not found in human urine but the pesticide chloropyrifos is metabolized to yield 3,5,6-trichloro-2-pyridinol.

Table 10: List of Chemicals Detectable in Human Urine, and Their Pesticide Origin

Chemical	Pesticidal Origin
	(Multiphenol Method)
Pentachlorophenol	Pentachlorophenol, lindane, and hexachlorobenzene
p-Nitrophenol	Methyl- and ethyl-parathion
2,4-D	2,4-D
2,4,5-T	2,4,5-T
Silvex	Silvex
3,5,6-Trichloro-2-pyridinol (3,5,6-TC-2-P)	Chloropyrifos
2,4,5-Trichlorophenol	2,4,5-Trichlorophenol, metabolite of certain organochlorine insecticides
Dicamba	Dicamba
	(Dialkyl Phosphate Method)
Dimethyl phosphate (DMP)	Any organophosphorus insecticide containing these phosphates or thiophosphates
Diethyl phosphate (DEP)	
Dimethyl phosphorothionate (DMTP)	
Diethyl phosphorothionate (DETP)	
Dimethyl phosphorodithionate (DMDTP)	
Diethyl phosphorodithionate (DEDTP)	

Table 11: Occurrence of Pesticide-related, Phenolic Residues in Human Urine[a]

Residue[b]	Percent positive	Arithmetical mean (ppb)	Maximum value (ppb)
Pentachlorophenol	84.8	6.3	193.0
3,5,6-TC-2-P	16.1	<5.0	31.7
2,4,5-TCP	1.7	<5.0	32.4
p-Nitrophenol	1.7	<10.0	113.0
Silvex	0.2	<5.0	3.2
2,4-D	0	-	trace
2,4,5-T	0	-	trace
Dicamba	0	-	-

[a]Based on the analysis of 416--418 samples collected from the general population via the Health and Nutritional Examination Survey II, National Center for Health Statistics.

[b]Limits of detection range from 5 to 30 ppb (5 to 30 µg/L).

Table 12: Occurrence of Dialkyl Phosphate Residues in Human Urine[a]

Dialkyl phosphate residue[b]	Percent positive	Arithmetical mean (ppb)	Maximum value (ppb)
Dimethyl phosphate (DMP)	11.5	<20	80
Diethyl phosphate (DEP)	7.9	<20	420
Dimethyl phosphothionate (DMTP)	6.5	<20	360
Diethyl phosphothionate (DETP)	10.8	<20	900
Dimethyl phosphodithionate (DEDTP)	0	—	trace
Diethyl phosphodithionate	0.2	<20	20

[a]Based on the analysis of 418 samples collected from the general population via the Health and Nutritional Examination Survey II, National Center for Health Statistics.

[b]Limit of detection for all residues = 20 ppb (20 µg/L)

D. Pharmacology and Metabolism of Silvex, Dicamba, and Related Pesticides

The fate of Silvex [2-(2,4,5-trichlorophenoxy)propanoic acid] was studied in seven men and one woman following oral administration of 1 mg/kg, and no adverse effects were observed (285). Recovery of Silvex and its conjugates in the urine through 168 h ranged from 66.6 to 95.1 percent of the dose administered, with a mean value and relative standard deviation of 80.3 ±10.5 percent. In humans, Silvex is readily absorbed after ingestion, and is subsequently readily excreted, predominantly *via* the urine (285).

The fate of 2,4,5-T following oral administration to humans has been reported (286); similar pharmacokinetic studies using ^{14}C-ring-labeled 2,4,5-T were conducted with other animals (287-291).

The pharmacokinetic data obtained with ^{14}C-ring-labeled Silvex indicated that a statistical projection of experimental results on large doses of Silvex to attempt to predict the hazard of exposure to small doses is most certainly not justified, because the capability to excrete Silvex in the urine becomes saturated. Urinary excretion of Silvex is saturated at the 50 mg/kg dose level (292).

The metabolic fate of ^{14}C-ring-labeled Dicamba (3,6-dichloro-2-methoxybenzoic acid) in animals has been reported (293). Following oral administration, the ^{14}C-labeled Dicamba was readily absorbed, but only partially metabolized. Within six hours after the final dose of Dicamba, ~89 percent had been excreted in the urine. The major metabolite was 3,6-dichloro-2-hydroxybenzoic acid (DCHBA). Minor metabolites identified in the urine were 2,5-dichlorophenol and the D-glucuronide conjugate of DCHBA. The fate of Dicamba has been studied in various components of the environment, including soils (294,295), plants (296-298), animals (293,299,300), and aquatic environment (293b).

Oral administration of 2,4-D, 2,4,5-T, or PCP to rats did not cause abnormal accumulation of porphyrin (*e.g.*, porphyrobilinogen) in the urine (301, 302), liver, or kidney (302), as observed for pentachloro- (303) or hexachlorobenzene (301). Similarly, 2,4,5-trichlorophenol (301,304), a metabolite of 2,4,5-T (305), did not effect any increase in total porphyrins in urine.

E. Trace Analyses of Pesticides: 2,4-D, 2,4,5-T, Silvex, Dicamba, and Others (General Overview)

For reliable assessment of human exposure to pesticides, and other toxic pollutants, analytical methods having a high degree of accuracy are required. In recent years, techniques have been developed to measure external exposure to pesticides in the urine; thin-layer chromatography, electron capture detectors for EC-GC, and, in some cases, gas chromatography--mass spectrometry are employed as confirmatory, analytical techniques.

Sample preparation is a very important factor for accurate analytical, trace analysis (281); the sample must be representative of the batch of material from which it was taken. Losses caused by absorption onto container surfaces, evaporation, thermal decomposition, or exposure to light can often be great enough to invalidate the results (306).

Solvent extraction is seldom specific for the analyte, and traces of impurities are often introduced from the solvent itself (281). There have been some recent improvements in the methodology of isolation and concentration; the use of organophilic resins, e.g., Amberlite XAD-2 and Tenax is noteworthy (306-308). The procedure for removing pesticides from urine with XAD-2 resin is rapid, requires no complex clean-up, and appears satisfactory for the extraction, and analysis (as methyl esters) of 2,4-D (and related herbicides) at the 0.1-ppm level in urine (269,309,310). Because 2,4,5-T, Silvex, and Dicamba are structurally quite similar to 2,4-D, and as they are largely unmetabolized in urine, the same technique can be used for their extraction and analysis.

There is a wide choice of pesticide-residue derivatives (311-313) and chemical derivatization methods (313,314) for chromatography, but only those capable of working efficiently on the microscale are acceptable. In view of the increased sensitivity of GC with electron-capture detection, (pentafluorophenyl)-methyl derivatives of acid herbicides have gained in importance, and have been applied in various fields (315-319).

Recently, DeBeer et al. (320) and Van Peteghem and Heyndrickx (312,322) reported a highly sensitive GC method, with mass-fragmentographic detection, for the quantitative determination of traces of 2,4-D in urine, using the trideuterated-methyl ester of 2,4-D as the internal standard. For the herbicides studied, e.g., 2,4-D and 2,4-DP [2-(2,4-dichlorophenoxy)propanoic acid], calibration curves in the nanogram range were established.

One of the main problems in pesticide-residue analysis is confirmation of the identity of the residue in the sample. This is essential and particularly difficult when no background information about the sample is available. Mass spectrometry and GC-MS are often the only techniques having a sensitivity sufficient for trace-analytical study. Modern, rapid-scanning, mass spectrometry, with on-line, computerized, data acquisition and processing, facilitates the collection of several thousand spectra during GC-MS analysis. These data can be further processed (mass-resolved chromatography), or be presented as a chromatographic profile, by using a particular ion or any selection of ions in the spectrum of complex mixtures (mass chromatography).

Modern methodology for organic trace-analysis provides many new techniques using spectrophotometry, paper- and thin-layer chromatography, and gas-liquid chromatography, combined with microcoulometric, flame-ionization, or electron-capture detection. Typical extracts submitted for analysis may require further purification in order to ensure that the compound of interest is free from substances that could cause chemical interference with its quantitative estimation. General-purpose detectors are invaluable in dealing with mixtures of unknown, volatile compounds, and the flame-ionization detector (FID) for GC has been extensively used in trace pesticide and food analyses. It is now possible to detect low-picogram quantities of 2,6-dimethylphenol with a FID (281).

The new improvements in GC methodology include the use of fused-silica columns (323,324), new capillary-column injector-design (325,326), the use of specific detectors (photoionization and ultrasonic), and IR-UV spectroscopic detectors, and also the use of mixed stationary phases.

The recently introduced negative-ion, chemical ionization (NICI), mass spectrometry (327-329) for quantitative estimation of electron-capturing compounds (e.g., toxic polychlorinated organics and pesticides (30,31,62,64, 330,331) offers unsurpassed sensitivity, and considerable selectivity. A recent application of negative-ion, atmospheric-pressure ionization (NIAPI), mass spectrometry for trace analysis offers a sensitive technique for determination of pesticides (e.g., 2,4,5-T) in blood and urine. The method gives quantitative data that differ to within only a few percent of the mean, for samples in the 50--100-pg range (332). The most selective detector is the thermal-energy analyzer (TEA) (333).

Modern methodology for trace analysis also includes liquid chromatography (LC) with UV detectors; (the technique is, however, much less sensitive than FID used in GC (334) liquid chromatography--mass spectrometry (LC-MS) (335); the use of a microbore column (336); also, chromatography with a mass spectrometer as the detector (selected ion-monitoring) (337); chemical ionization (338, 339); metastable-ion, mass spectrometry (340), e.g., mass-analyzed, ion, kinetic-energy spectrometry (341), and immunological assays (342,343) as an alternative to mass spectrometry.

F. Additional Recent Results on Analysis of Pesticides

A multiresidue procedure for the determination, and confirmation, of a variety of acidic herbicide residues in human urine has recently been described by Draper (344). 2,4-D, 2,4,5-T, Silvex, Dicamba, PCP, and others were determined simultaneously with a high degree of quantitative accuracy, precision, and sensitivity. Samples were hydrolyzed with mineral acid in order to liberate the residues from conjugates. Acids were isolated from the urine hydrolyzate by acid--base partitioning, and derivatized with ethereal diazomethane. Pesticides were determined quantitatively by EC-GC, and structures were confirmed by computer-controlled GC-MS. Recoveries were 80--104 percent for fortifications at 0.1 mg/L, and detection limits for herbicides in urine were 0.05--0.1 mg/L by EC-GC, and 0.1--0.5 mg/L by GC-MS. Methylation of urinary extracts was found superior (344) to (pentafluorophenyl)methylation (318). Derivatization with (pentafluorophenyl)methyl bromide (PFB) was unacceptable for several reasons: it enhanced the electron-capture response of urinary acid herbicides, and the specific detection of PFB analogs by mass spectrometry was limited by the similarity of their electron-impact, mass spectra. Exposure of forest workers to 2,4-D was carefully monitored; human exposure-levels in these tests were below that which might endanger health (23.7--57 µg/kg of body weight) (345). The lowest detectable concentration of 2,4-D and 2,4,5-T in human urine was (346) 0.05 mg/mL. Mass-fragmentographic determination of 2,4-D in human urine, using deuterated, internal standards, has been discussed (320). Separation and identification of 2,4-D (347) and other chlorinated pesticides (348) in urine by thin-layer chromatography have been described. The authoritative, recent book by Lawrence (348a) deals with the application of HPLC to the analysis of pesticides, e.g., fungicides, herbicides, plant growth regulators, and rodenticides.

VII. PHTHALATE ESTERS (PLASTICIZERS) IN HUMAN URINE

A. General

Esters of phthalic acid are widely used as industrial solvents and plasticizers in the manufacture of a variety of plastic formulations. The use and production of phthalic ester plasticizers, e.g., bis(2-ethylhexyl) phthalate (DEHP), bis(2-ethylhexyl) adipate (DEHA) and diethyl (DEP) or dibutyl (DBP)-phthalate have been steadily increasing over the past two decades. The total annual production has reached nearly 5×10^8 kg and continues to increase (349-353). The plasticizer industry predicts future yearly increases of 6 percent in plasticizer production in the 1980's (354).

The most widely used plastic in medical devices is poly(vinyl chloride) (PVC). To make the rigid PVC more flexible, plasticizers are added. These plasticizers may contribute up to 40 percent by weight of the finished product (355,356). DEHP is the plasticizer most commonly used in medical devices.

The main use of diethyl phthalate (DEP) as a plasticizer is in the production of cellulose acetate films, which may contain up to 20 percent of the finished product. In addition, it is used extensively as a denaturant for cosmetic alcohol and in hair-spray preparations (357).

The plasticizer DEHP has achieved increased interest; together with other phthalate esters, it constitutes a new class of environmental pollutants (349, 357,358). Over one-fourth of the total production of plasticizers in 1972 was accounted for by DEHP. In addition to animal and human urine (350,351), DEHP has been found in commercial solvents (359), the organic matter of soil (360), water (361), marine water (349), ocean sediment (349,353), fish (362), fatty foods taken from plastic packages (363), automobile atmospheres (364), milk (365), rice meal, herbs, instant coffee, and cocoa (366), and human blood (367-370). Phthalate ester plasticizers were found to be extracted by blood from plastic tubing and from the plastic bags used for blood storage (368); the plasticizers were found in patients who had received extensive blood transfusions (368,371). Plasticizers were also found in human plasma (372), platelet concentrations (373), and human tissue (374,375). There are reports that phthalates are formed during the thermal oxidation of cooking oils (376), and there is even some evidence that indicates that they occur naturally as a result of biosynthesis (377,378). A recent report (349) indicates a high level of phthalate esters in the water near the Gulf of Mexico ($\sim$100 ng/L), but a relatively low level in fish samples ($\sim$5 ng/g). This can be interpreted as the possible consequence of metabolism and excretion by the fish, and warrants monitoring for phthalate metabolites, as well as for the parent compounds.

The leaching of phthalates from hemodialysis equipment was demonstrated, and its implication in the production of hepatitis in blood-transfusion patients has been considered (356,379-381).

B. Toxicity of Phthalate Esters

Autian (356) reviewed the toxicology of the phthalate plasticizers in animals and in cell culture. Phthalate esters have been reported to have a low order of toxicity (356,382-390) and a weak teratogenic effect (391-393) in experimental animals. However, Guess et al. (394) emphasized the subtle toxic effects of phthalate ester plasticizers. Recent results (395) suggest that the effects of phthalate esters (e.g., DEP) arise from the toxicity of their metabolites.

Diethyl phthalate (DEP) has been shown to be toxic to cells in culture systems, and to be teratogenic in the chick embryo (396) and rat (391), and, when it is added (5 percent) to the diets of male rats, it causes an increase in the liver weight (357). Further studies are required in this area of subtle toxic effects, possibly associated with accumulation and metabolism of phthalate esters (389).

a. Toxicity of DEHP

The extensive use of polyvinyl-stored blood and blood-products during the past decade has failed to reveal any definitive adverse effects in humans of such storage. The results presented by Rubin and Schiffer (355) and Wallin et al. (387) indicate rapid clearance of DEHP from blood, and extensive urinary excretion within 24 hours; this may be seen from Table 13.

Table 13: Urinary Excretion of DEHP (355)

		24-hr DEHP excretion (mg DEHP equivalents)		
Patient #	Total DEHP received (mg)	Pre-transfusion	Post-transfusion	Max. excreted (%)
3	62.2	9.0[a]	55.9[b]	90
4	67.8	13.8	40.0	60[c,d]

[a]A 12-hour collection immediately prior to transfusion: 1250 mL of urine.

[b]Not a complete 24-hour collection, but does contain all of the urine excreted during the first 8 hours, and most of the urine excreted during the subsequent 16 hours: 1650 mL of urine.

[c]The urinary data indicate that a major portion of the DEHP administered is excreted within 24 hours, although determination of the exact quantitative portion is complicated by the pretransfusion excretion of significant amounts of DEHP-related material.

[d]The assay procedure used for the urine samples does not distinguish between unchanged DEHP and phthalic acid-containing metabolites.

Thus, the results (355,397) do not support any concern that the transfusion of blood stored in poly(vinyl chloride) blood-bags leads to deposition of the plasticizer, DEHP, in the tissues of man. However, these facts should not diminish the concern for any possible adverse biological effects of acute or chronic exposure to this compound, particularly when one considers that patients in a wide array of debilitated states receive transfusions of blood and blood products. The authors (355) advocated the development of alternative plastic formulations that do not result in appreciable migration of any chemical constituents. DEHP has been shown to be detrimental to the reproduction of marine organisms at parts per billion (ppb) levels in water (398). Studies recently reported by the National Toxicology Program identified DEHP and bis(2-ethylhexyl) adipate (DEHA), as hepatocarcinogens in mice (399).

C. Metabolism of Phthalate Esters

a. General

The leaching of DEHP by blood but not by simple salt solutions (368), from plastic transfusion bags (369) and medical-grade hemodialysis tubings (400) has been demonstrated. Lewis et al. (401) (401) reported that 6.1 mg of DEHP was leached into blood in 1 hr in patients undergoing hemodialysis therapy. Later, Chen et al. (402) found that the 24-hr urinary DEHP concentrations of post-dialysis urines range from 0.9 to 2.6 percent of the total 6.1 mg of DEHP that migrated from the hemodialysis system. The low concentrations of DEHP excreted in the urine suggest that DEHP leached into patients during hemodialysis treatments could be taken up by tissues or be metabolized. (Compare Table 13.) The detection limit of DEHP in urine is (402) 15 ng/mL. Lewis et al. (401) also reported that most of the DEHP present in serum at the completion of hemodialysis will disappear in 5 to 7 hours. The foregoing data thus amplify existing knowledge concerning the metabolic disposition of DEHP, and reinforce the current opinion that the use of this material does not pose an imminent threat to human health.

b. Metabolism of DEHP

Shaffer et al. (382) reported for the first time on metabolic studies of DEHP in man and rabbits as early as 1945. However, knowledge concerning the metabolic aspects of DEHP remained scanty until detailed metabolic studies were made in 1972.

Papers on the metabolism of DEHP published since 1972 by many workers (368, 403-410) describe in detail the elimination, distribution, urinary metabolites, and hydrolysis of DEHP, in many cases (403-405,411) with respect to experimental animals. Five urinary metabolites of DEHP, including mono(2-ethylhexyl) phthalate (MEHP) and phthalic acid were confirmed (403,406); another report demonstrated the presence of seven (397), and more-recent findings (412) even nine, metabolites of DEHP. As shown by Albro et al. (406) and by Daniel and Bratt (403), DEHP following oral administration is rapidly hydrolyzed to the half-ester, MEHP, by pancreatic lipase; the principal metabolites being identified as the acid, alcohol, and ketone resulting from the sequential ω- and (ω-1)-oxidation of MEHP. The alcohols can be further oxidized to the corresponding carboxylic acid or ketone and the keto-acid may subsequently be subject to β-oxidation.

Human leukemia patients were recently reported (404,405) to excrete 80 percent of their DEHP metabolites in urine as D-glucuronide conjugates; in the 24-hr urine of these patients, MEHP was a significant metabolite (20 percent); alcoholic metabolites amounted to >60 percent and diacids constituted only ~9 percent of the total.

Elimination, distribution, and metabolism of DEPH in animals were also studied by the tracer technique, using [^{14}C]carbonyl DEHP (387,403,410). Of the dose, ~80 percent was excreted in the urine in 5 to 7 days following intravenous or oral administration; the [^{14}C]DEHP was preferentially localized in the liver for a short time (387,403,406). The transformation of DEHP into certain metabolites in aquatic organisms has been reported (413-415).

c. Metabolism of related phthalate esters

The urinary metabolites following oral administration of dimethyl (416), dibutyl (416,417), or dioctyl (416) phthalates have been reported. When administered into the stomach, most of the diethyl phthalate (DEP) and dibutyl phthalate (DBP) was metabolized to monoester and phthalic acid, and these were rapidly excreted in the urine (395). Exposure of rats to DBP aerosols (concentration 50 mg/m^3, 6 hours daily for 6 months) has also been examined (392); the results suggest that the adverse effects of phthalate esters arise from their accumulation in the brain. The *in vivo* metabolism of dialkyl phthalates has been studied extensively (356,357,406,410).

D. Determination of Plasticizers in Human Urine

The methods applied for the determination of phthalate esters in human and animal urine involve an array of techniques, including GC, LC, GC-MS, and computerized mass spectrometry. Recent analytical methodology and techniques have been comprehensively reviewed by Risby *et al.* (418) (GC), Majors *et al.* (419) (LC), and Burlingame *et al.* (420) (GC-MS and GC-MS-on-line computer techniques).

a. GC methods

The original GC method of Piechocki *et al.* (372) for the determination of DEHP in human plasma (limit of detection, 50 ng of DEHP) has been widely applied for the analysis of phthalates in urine. In a typical procedure (421), DEHP and all phthalic acid-containing metabolic intermediates of DEHP in the urine were hydrolyzed to phthalic acid by the method of Shaffer *et al.* (422) as previously described (374). Following methylation with diazomethane, the phthalic acid content was determined by GC (of the dimethyl phthalate formed) in a 6 ft column of 3 percent SE-30 using a flame-ionization detector. Hexadecane (C_{16}) was used as the internal standard for quantitation. Other GC methods (355,402), or a combination of GC, t.l.c., and spectroscopy techniques (406) for analysis of phthalates in urine have been described. Recently, Peterson and Freeman (353) have reported a highly sensitive and selective GC-MS analytical scheme (423) for the analysis of phthalate esters (*e.g.*, DEHP) found in dated sediment cores of the Chesapeake Bay. The GC-MS method (395,405,406,416) thus proved to be efficient for the quantitative analysis of phthalate esters and their metabolites in urine.

b. LC methods

Recently, Draviam et al. (424) developed a method for the separation and quantitation of plasticizers (e.g., DEHP) and their metabolites from human urine using liquid chromatography (LC). Urine was diluted with an equal volume of water, and extracted at pH 2 with diethyl ether. The extract was dried, the solvent vacuum-stripped, and the residue dissolved in methanol for injection into the chromatograph. A C_{18}, reversed-phase column containing 10-μm particles was used for the analysis. Ionic suppression, 0.5 percent acetic acid in water, at pH 3 was used to resolve the acidic components. A step gradient of acetonitrile--water (containing acetic acid) was used to elute the polar metabolites as well as the nonpolar plasticizers. Mass spectrometry was used to identify the compounds in the LC fractions. From the LC fractions of the urine extract phthalic acid, MEHP, DEHP, and normal urinary constituents (e.g., hippuric and benzoic acid derivatives) were identified. The detection limits (at a wavelength of 245 nm) for a reference mixture of phthalic acid and phthalate esters are listed in (424) Table 14.

Table 14: Detection Limits for Phthalate Esters[a]

Compound	Detection limits (μg/mL)
Phthalic acid	2.7
Monomethyl phthalate	1.5
Monobutyryl phthalate	3.3
Monobutyl phthalate	1.6
Mono(2-ethylhexyl) phthalate	2.1
Butyl benzoyl phthalate	2.0
Bis(2-ethylhexyl) phthalate (DEHP)	1.4

[a]LC, UV detector at 245 nm.

c. Recent LC-MS methods

Recently, Harvan et al. (412) discussed, and compared, the electron impact (EI), methane-positive chemical ionization (MPCI), oxygen-negative chemical ionization (ONCI), and collision-induced dissociation, mass-analyzed, ion kinetic energy (CID-MIKE) spectra (208,414,425,426) of the nine metabolites of DEHP isolated from rat urine. The metabolites were methylated with diazomethane, and fractions were purified by LC on a Porasil A-60 column using a tetrahydrofuran--hexane gradient (prefractionation is necessary).

The DI mass spectra of all metabolites of DEHP are very similar, so that little specific structural information is afforded by this technique. The MPCI and ONCI techniques give similar information, in that the mass of the side-chain can be deduced in each case from the respective mass spectrum. However, the data are complementary in the sense that information gained by the observation of the loss of a fragment in one case is usually available by formation of that ion in the other; this is exemplified by the formation of the side-chain ion in the ONCI spectra. Thus, either method of ionization can be used as a check on the other. This is further illustrated by the ion--molecule reaction-product ions observed in the case of the hydroxyl metabolites. The presence of the hydroxyl group is indicated by the $[MH-H_2O]^+$ ions observed in the MPCI spectra, and is supported by the $[M + O_2]^-$ ions found in the ONCI experiment.

The CID--MIKE spectra demonstrated the potential of this technique as a versatile and specific detection-system. Although the EI, MPCI, and ONCI mass spectra of the two isomeric tri-esters [e.g., 1,2-dialkyl esters of 3-alkyl-o-phthalic acid, metabolites of DEHP) (406) were similar, their MIKE spectra were different. This enables the use of single reaction monitoring, e.g., the monitoring of a particular fragment-ion generated from a specific parent-ion and application of this information for determination of the structure. The MIKE spectra were thus used to assign the structure for four new, including three hydroxylated, metabolites of DEHP (412). The other methods used for analysis involve the radio-assay of ^{14}C-labeled DEHP (and metabolites) in urine, followed separation by t.l.c. (387,389,427).

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This survey reviews and discusses the occurrence of priority pesticides and industrial chemicals in human urine. An overview of some recent analytical methodology for determination of selected toxic pollutants and their metabolites as they are found in human urine is also presented. The review includes 427 references.

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